Fundus Autofluorescence Imaging

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Core Messages

- With the advent of confocal scanning laser ophthalmoscopy, fundus autofluorescence (FAF) intensity and distribution can be recorded in vivo
- FAF imaging gives information over and above conventional fundus photography and fluorescence angiography and is a noninvasive diagnostic tool for evaluating age- and disease-related alterations of the retinal pigment epithelial (RPE) layer
- The FAF signal derives from fluorophores in lipofuscin granules within the RPE cell cytoplasm, with A2-E being a dominant fluorophore
- RPE lipofuscin accumulates with age and represents a common downstream pathogenetic pathway in various monogenetic and complex retinal degenerations
- Absorbing structures anterior to the RPE including retinal vessels and macular pigment as well as lack of autofluorescent material in RPE atrophy are associated with a decreased autofluorescence signal
- Topographic patterns of abnormal FAF may vary considerably in eyes with similar manifestations on funduscopy. Therefore, FAF imaging allows for more precise phenotyping
- For age-related macular degeneration it has been shown that particular FAF phenotypes have an impact on disease progression
- Findings of FAF imaging in retinal degenerations underscore the pathophysiological relevance of potentially toxic properties of excessive lipofuscin accumulation in the RPE
- Visualizing metabolic changes in RPE cells may be helpful for monitoring novel interventional strategies aimed at slowing accumulation of toxic lipofuscin compounds
- High-resolution cSLO fundus autofluorescence imaging now allows for visualization of the polygonal RPE cell monolayer with delineation of individual cells in vivo

2.1 Introduction

2.1.1 Advances in Ocular Imaging: Visualization of the Retinal Pigment Epithelial Cell Layer

Retinal pigment epithelial (RPE) cells possess numerous functions which are essential for normal photoreceptor function. The RPE cell monolayer has also been implicated in various retinal diseases [1, 21, 51, 57]. Given the close anatomical relationship to layers posterior and anterior to the RPE cell monolayer, postmitotic RPE cells are involved in disease processes even if the specific cause originates, e.g. from cells of the neurosensory retina or the choroid. Given the crucial role in retinal disease, various attempts have been made to visualize the RPE in the living eye. While fluorescence angiography mainly detects secondary effects such as alterations in the outer blood-retinal barrier, resolution, e.g. of ultrasonography or optical coherence tomography, was insufficient to visualize the cellular elements. With the advent of confocal scanning laser ophthalmoscopy, which was initially developed by Webb et al. [56], it is now possible to record fundus autofluorescence (FAF) and its spatial distribution in vivo (Fig. 2.1). Therefore FAF imaging represents a diagnostic, noninvasive tool for evaluating the RPE during ageing and in ocular disease. As shown by spectrometric findings by Delori et al. [17], the FAF signal mainly derives from RPE lipofuscin. Methodological developments with higher resolution now even allow for delineation of individual RPE cells in the human eye. Spaide has described a method by which autofluorescence photographs can be obtained using a fundus camera-based system [50].

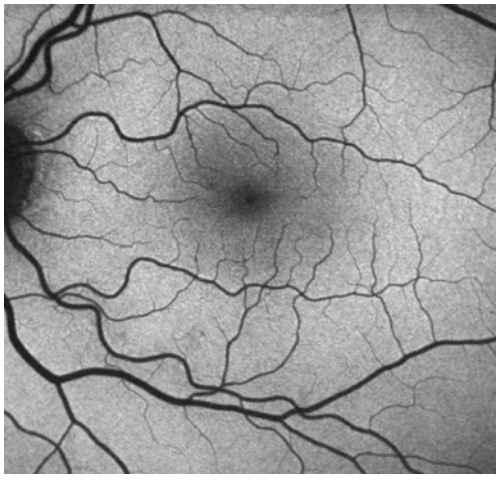

Fig. 2.1. FAF mean image of a 59-year-old male patient with normal topographic distribution of FAF intensity. Absorption by macular pigment and by retinal vessels results in decreased FAF signal intensitiy

2.1.2 Lipofuscin Accumulation in the RPE Cell: A Common Downstream Pathogenetic Pathway

An essential function of postmitotic RPE cells is the lifelong phagocytosis of shed photoreceptor outer segment discs and degradation with subsequent release of degraded material at the basal cell side, where it is normally cleared by the choriocapillaris. With age lipofuscin accumulates in the lysosomal compartment [17, 23]. It is also known to present a common pathogenetic pathway in various monogenetic and complex retinal diseases and is associated with photoreceptor degeneration. Although the mechanisms of lipofuscinogenesis are incompletely understood, there is strong evidence that oxidative damage plays an important role, with antioxidant deficiency or oxidant conditions being of importance [2, 4, 15].

Several lines of evidence indicate that lipofuscin is not an inert by-product but

that it interferes with normal cell function and that it may cause cell death upon reaching critical concentrations. Recent analyses of molecular compounds in isolated human lipofuscin granules revealed various molecules with *toxic properties* including lipid peroxidation products [27], protein alterations in association with malondialdehyde (MDA), 4-hydroxynonenal (HNE) and advanced glycation end products (AGE) [49] as well as a Schiff base reaction product, *N*-retinylidene-*N*-retinylethanolamine (A2-E) [22]. A2-E represents the dominant fluorophore of lipofuscin in the RPE. But other fluorophores that occur in association with retinal diseases must be considered when interpreting FAF images including fluorophores in subretinal fluid or blood components from haemorrhages.

Molecular mechanisms have elucidated how A2-E interferes with normal lysosomal function [7, 28, 48]. Further evidence for a pathophysiologic role of lipofuscin includes a similar topographic distribution of lipofuscin and drusen, accelerated accumulation of lipofuscin in monogenetic macular dystrophies such as Best or Stargardt disease and a striking deposition of A2-E in RPE cells in ABCR knockout mice with strong dependence on light exposure. Furthermore, A2-E possesses phototoxic and detergent properties and is capable of inducing disintegration of various organelle membranes upon reaching a critical concentration [48].

Summary for the Clinician

- **Lipofuscin granules accumulate in the RPE with age and in association with various retinal diseases**
- **Lipofuscin contains toxic compounds including A2-E and lipid peroxidation products which interfere with normal cell functions upon reaching critical levels**
- **The fundus autofluorescence signal mainly derives from lipofuscin fluorophores in the RPE as shown by spectrometric analyses [17]**
- **The retinoid A2-E is the dominant fluorophore in lipofuscin granules**

2.1.3 Confocal Scanning Laser Ophthalmoscopy for Fundus Autofluorescence Imaging

Information on lipofuscin accumulation in the RPE has been largely obtained in vitro from studies using fluorescence microscopy techniques and in vivo from fundus spectrophotometric investigations [17]. Recently, with the advent of *confocal scanning laser ophthalmoscopy* using appropriate excitation wavelengths and barrier filters, it is now possible to record topographic variations of lipofuscin-related autofluorescence in vivo. The technique was initially introduced by von Rückmann and co-workers using a Zeiss SLO prototype [52]. A commercially available confocal SLO (Heidelberg Retina Angiograph, HRA, Heidelberg Engineering) has subsequently been used for FAF imaging with an adequate excitation wavelength (argon 488 nm in the HRA classic or an optically pumped solid state laser at 488 nm in the HRA2) and a barrier filter to detect emission from dominant RPE lipofuscin fluorophores above 500 nm [5, 10, 26]. The optical and technical principles of the HRA have been described previously [25, 26]. Maximal retinal irradiance using the HRA is approximately 2 mW/cm² for a 10×10° frame and is, therefore, well below the limits established by the American National Standards Institute and other international standards (ANSI Z136.1-2000).

One of the difficulties encountered during FAF imaging besides careful and stan-

dardized image acquisition is the influence of media opacities, with cataract being the most prominent adverse factor. Therefore, image quality may vary considerably depending on lens opacity. In the multicentre FAM Study (*F*undus *A*utofluorescence in Age-Related *M*acular Degeneration Study), a standard operation procedure has been proposed, which includes focussing in reflectance and redfree mode, acquisition of at least 15 single 30° images, automated alignment and calculation of a mean image out of about 9 single images to amplify the signal to noise ratio [43].

Summary for the Clinician

- **Using adequate excitation wavelengths and barrier filters scanning laser ophthalmoscopy allows for detection of topographic and spatial distribution of fundus autofluorescence in vivo**

2.2 Autofluorescence Imaging in Retinal Diseases

2.2.1 Autofluorescence Imaging in Age-Related Macular Degeneration

Age-related macular degeneration (AMD) has become the most common cause of legal blindness in all industrialized countries [12, 13, 31]. Several lines of evidence indicate that the RPE cell layer plays an important role in the pathogenesis of both early and late manifestations. Drusen represent a hallmark of the ageing retina and early AMD. Their composition includes incompletely degraded material from autophagy and phagocytosed shed photoreceptor outer segment discs. Given the similarities between topographic lipofuscin and drusen distribution and the implication of lipofuscin formation and lysosomal dysfunction, it is assumed that lipofuscin plays a pathogenetic role in AMD. This hypothesis is further underscored by the observation of excessive lipofuscin accumulation in juvenile macular dystrophies [54] and the fact that excessive lipofuscin accumulation has been shown to precede geographic atrophy [26]. There is additional experimental evidence for adverse effects of lipofuscin [29]. Therefore, the application of FAF imaging in patients with AMD appears particularly attractive to further elucidate processes.

In Germany, a prospective multicentre natural history study (*F*undus *A*utofluorescence in Age-Related *M*acular Degeneration, FAM Study) was initiated and the results are reported in the following sections.

2.2.1.1 Geographic Atrophy

In eyes with geographic atrophy due to AMD, various different patterns of abnormal FAF were noted at the posterior pole outside the actual atrophic patches. These were classified into banded, patchy, focal and diffuse patterns. The latter type was further differentiated into the following subtypes: reticular, fine granular, branching and peripheral punctate [11, 45]. Hereby many alterations were only seen on FAF images without corresponding funduscopically visible alterations. It is assumed that these patterns may reflect heterogeneity on the molecular level and may, therefore, represent different disease entities. The classification may therefore be helpful to identify specific genetic or environmental factors. Interestingly, recent analyses have also shown that different FAF patterns in the junctional zone of geographic atrophy have an impact for disease progression, and may therefore serve as novel prognostic determinants for the enlargement of geo-

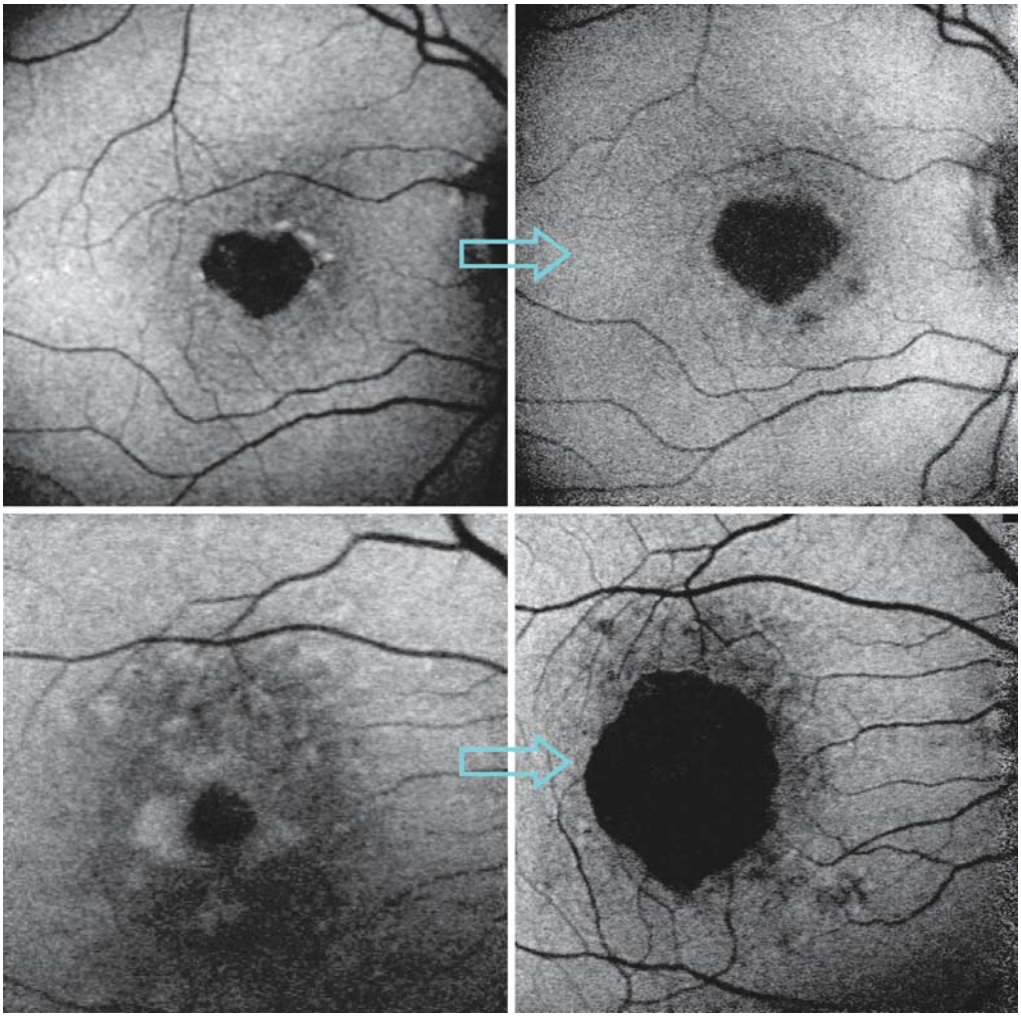

Fig. 2.2. Spread of atrophy over a 1-year period. While the pattern of focal increased FAF in the junctional zone of geographic atrophy shows only little enlargement (*top*), marked spread occurs in the presence of larger areas of elevated FAF outside the atrophic patch (*bottom*)

graphic atrophy over time and progressive visual loss (Fig. 2.2) [8].

Longitudinal observations have also shown that areas with increased FAF, and therefore excessive RPE lipofuscin, in the junctional zone of geographic atrophy precede the enlargement and development of new atrophic patches over time [26]. Such areas may therefore be regarded as incipient atrophy (Fig. 2.3).

Besides imaging increased levels of FAF due to a higher content of RPE cell lipofuscin (Fig. 2.4), FAF imaging is also a very accurate method for identifying and delineating areas of geographic atrophy which due to absence of autofluorescent RPE are associated with a corresponding markedly decreased FAF signal. The method is superior for this purpose to conventional imaging methods such as fundus photographs

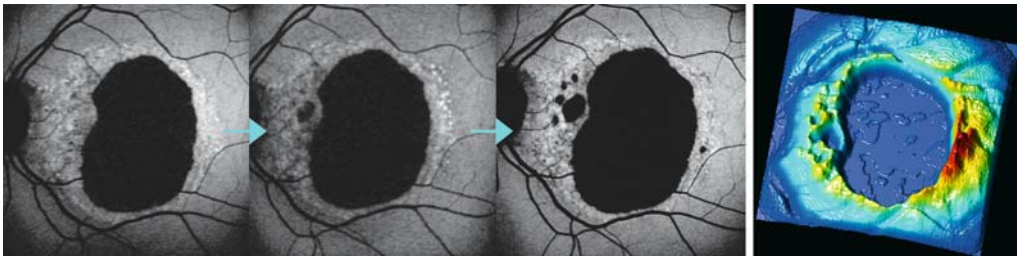

Fig. 2.3. Over time, enlargement of existing atrophy and occurrence of new atrophic patches due to age-related macular degeneration occurred only in areas with abnormally high FAF at baseline, reflecting the pathophysiological role of excessive lipofuscin accumulation in RPE cells [26]

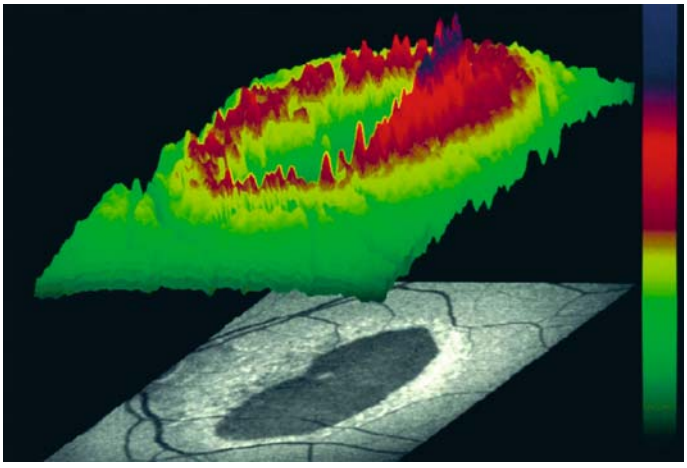

Fig. 2.4. Colour coded intensities of FAF signals. There is an increased FAF in the junctional zone of a kidney-shaped patch of geographic atrophy associated with age-related macular degeneration

or fluorescein angiography. In addition the digital images are readily available for quantitative measurements, whereby software has been developed to allow for partially automated detection of atrophic areas [16, 43]. This method can now be used for following patients with geographic atrophy and particularly in clinical trials with interventions to slow down enlargement of atrophic patches.

Despite obvious interindividual variations a high degree of intraindividual symmetry has been noted not only for the distribution of atrophic patches but also for the abnormal FAF in the junctional zone using FAF imaging [5].

Summary for the Clinician

- **Areas of geographic atrophy are characterized by a low FAF signal due to lack of autofluorescent material at the level of the RPE, which allows for precise delineation of atrophic patches and their enlargement over time**
- **Atrophic areas due to AMD are surrounded by various different patterns of abnormal FAF with extensive interindividual variability and a high degree of intraindividual symmetry, which may reflect heterogeneity on a molecular level**
- **The pattern of abnormal FAF in the junctional zone has an impact on disease progression**

- **Increased FAF precedes enlargement of pre-existing atrophy and development of new areas of geographic atrophy**

2.2.1.2 Drusen

With regard to the FAF signal from individual drusen, it may be increased, normal to background fluorescence or decreased. While drusen in association with juvenile macular dystrophies tend to show an increased FAF, drusen due to AMD rather have no abnormal or a decreased FAF signal [55]. Both composition of drusen material and/or alterations of the overlying RPE may account for these phenomena. Concurrent focal or linear hyperpigmentations in eyes with drusen are usually associated with an increased FAF signal, which is thought to derive from melanolipofuscin [53].

Together with the pooled images of the FAM Study centres and two additional centres (Moorfields Eye Hospital, Institute of Ophthalmology, London; Department of Ophthalmology, University of Brescia, Italy), FAF changes were classified in eyes with early AMD and absence of late atrophic or neovascular manifestations into eight phenotypic patterns including normal, minimal change, focal increased, patchy, linear, lace-like, reticular and speckled [9].

Interestingly, the FAF changes do not necessarily correlate topographically with visible fundus changes in patients with early AMD. Areas of increased FAF may or may not correspond with areas of hyperpigmentation, soft or hard drusen. The FAF signal may be normal, decreased or increased in corresponding drusen areas. This may reflect the variable composition of drusen including other fluorophores as well as different reactive alterations in the overlying RPE cell monolayer. Overall, larger drusen were associated more frequently with more pronounced FAF abnormalities than smaller ones. Areas covered with so-called reticular drusen, or reticular 'pseudodrusen' as termed by others [3, 32, 36], usually show a unique reticular FAF pattern with multiple small, uniform areas of decreased FAF surrounded by normal FAF (Fig. 2.5).

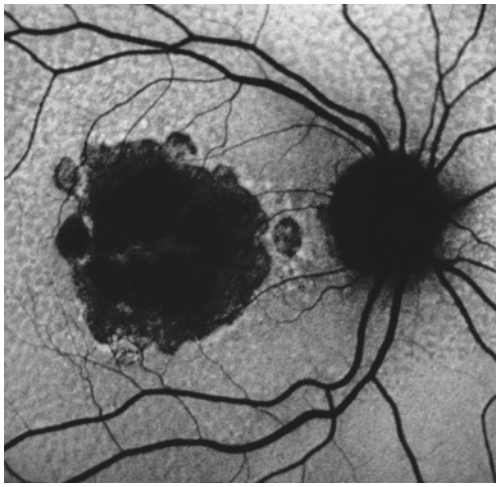

Fig. 2.5. FAF mean image of a patient with central geographic atrophy due to age-related macular degeneration. Outside the atrophic patch a typical reticular pattern corresponds to funduscopically visible 'reticular drusen'

Delori et al. have reported that soft drusen may display an annulus of increased FAF [18]. Possible explanations are: (1) that the RPE is somehow stretched over a discrete druse and therefore might contain a thinner layer of lipofuscin granules, (2) that the druse causes the central overlying RPE to release lipofuscin, which is phagocytosed by RPE at the border of the druse and (3) that drusen are formed as a consequence of incipient RPE atrophy. However, FAF changes remote from funduscopically visible alterations may indicate more widespread abnormalities and diseased areas. It may be speculated that changes seen with FAF imaging on the RPE cell level may precede the occurrence of funduscopically visible lesions as the disease progresses. Fur-

ther longitudinal studies will be needed to test the hypothesis that different phenotypic FAF variations in eyes with drusen are of prognostic relevance.

Summary for the Clinician

- **Drusen can be associated with increased, decreased or normal FAF**
- **This may reflect heterogeneity both in the composition of drusen material basal to the RPE or variable alterations of the overlying RPE**
- **Drusen are associated with variable abnormal FAF changes in retinal areas remote from the drusen**
- **Study results are pending with regard to the prognostic relevance of these findings**

2.2.1.3 Pigment Epithelial Detachments

Observations in eyes with pigment epithelial detachments (PEDs) due to AMD, idiopathic central serous chorioretinopathy or polypoidal choroidal vasculopathy (PCV) suggest that funduscopically and angiographically similar appearing PEDs are associated with variable FAF phenomena. Interestingly, the corresponding area may have a markedly decreased, increased or normal FAF signal (Fig. 2.6). These variations in FAF may reflect different stages of evolution in the development of PEDs which typically enlarge over time, then flatten or turn into a RPE tear, and, finally, disappear with a subsequent corresponding area of geographic atrophy or fibrovascular scarring associated with irreversible loss of

Fig. 2.6 A–C. FAF **A**, early **B** and late **C** phase of fluorescein angiography in a 77-year-old patient with a large pigment epithelial detachment due to age-related macular degeneration

neurosensory retinal function. Preliminary observations indicate that PEDs in younger patients, e.g. due to idiopathic central serous chorioretinopathy, usually show an increased autofluorescence signal. Furthermore, there is frequently a halo of decreased FAF at the margin of the PED, which is thought to originate from absorption effects of subneurosensory extracellular fluid [42].

FAF changes in the presence of PEDs may not only result from LF granules in the RPE. The extracellular fluid between the detached RPE and Bruch's membrane may also contain fluorophores which show up in the excitation and emission range applied for FAF imaging. However, these molecular species are currently unknown and remain to be identified.

Summary for the Clinician

- **FAF findings corresponding with PEDs change over time and may relate to the evolution of the disease process with enlargement, flattening and disappearance of the detachments**
- **An increased FAF signal in the presence of PEDs may also originate from the subpigment epithelial extracellular fluid, whereby the fluorophores are yet unknown**

2.2.1.4 Correlation of cSLO Microperimetry and Fundus Autofluorescence

Normal photoreceptor function requires normal RPE cell function and in particular the constant phagocytosis of photoreceptor outer segment (POS) discs by the RPE. If excessive lipofuscin accumulation inhibits this degradative metabolism, the rate of phagocytosis of POS discs would be impaired, which would, in turn, induce abnormal photoreceptor function. Using scanning laser ophthalmoscopy in combination with macular microperimetry, it is possible to test retinal sensitivity precisely over areas of abnormal FAF [39, 41]. We have shown that areas of increased FAF in the junctional zone of geographic atrophy are associated with variable degrees of retinal sensitivity loss, which would indeed indicate a functional correlate of excessive RPE lipofuscin accumulation in AMD [44]. Scholl et al. (2004) have demonstrated that increased FAF is associated rather with scotopic than with photopic sensitivity loss [46]. These findings underscore the potential pathophysiologic role of lipofuscin accumulation in the RPE.

Summary for the Clinician

- **Increased FAF tends to be associated with corresponding impaired neurosensory retinal function as shown by combining cSLO microperimetry and FAF imaging**

2.2.2 Fundus Autofluorescence Imaging in Macular and Retinal Dystrophies

In macular and retinal dystrophies various changes in FAF have been described [55]. In Best disease, adult vitelliform macular dystrophy and Stargardt macular dystrophy-fundus flavimaculatus yellowish-pale deposits at the level of RPE/Bruch's membrane are associated with markedly increased FAF intensity [17, 54]. In Stargardt macular dystrophy focal flecks typically show bright, increased FAF and may fade as atrophy develops. This reflects abnormal regions of RPE engorged with abnormal lipofuscin-like material. By way of contrast in patients with Stargardt macular dystrophy-fundus flavimaculatus, Lois et al. described – besides high FAF – also normal or low FAF intensities [35]. Low levels of FAF in such patients were associated with pe-

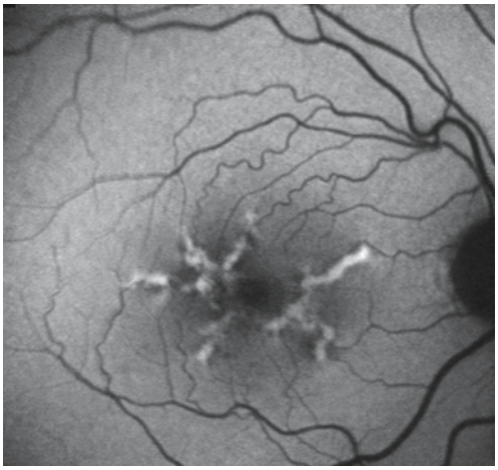

Fig. 2.7. Typical radial lines of increased FAF in a patient with a pattern dystrophy

ripheral cone and rod dysfunction (ERG) whereas patients with normal or high levels of FAF had normal peripheral cone and rod function. There was no relationship between levels of FAF and macular dysfunction. Different FAF patterns in patients with vitelliform macular dystrophy have been described as 'spokelike', 'diffuse' or a combination of both [14].

The abnormally intense FAF – also seen in pattern dystrophies (Fig. 2.7) – suggests a generalized abnormality of the RPE. Additionally the so-called dark choroid (lack of choroidal fluorescence) in some macular dystrophies implies a retinal pathology and might be due to different fluorophores in different disorders. However, in some patients of families with known pattern dystrophy due to a mutation in the *rds* gene, normal fundus morphology and no functional deficit in electrophysiology and psychophysics was associated with increased levels of FAF [55]. Additionally FAF changes can occur in patients with hereditary retinal degenerations that are associated with extraocular changes. In 1959, Kjellin described an autosomal recessive syndrome with spastic paraplegia, mental retardation, amyotrophia, and 'central retinal degeneration' [30]. In another case with Kjellin's syndrome published in 2002 [24], biomicroscopy disclosed symmetric multiple round yellowish flecks at the level of the retinal pigment epithelium scattered at the posterior pole, which showed increased

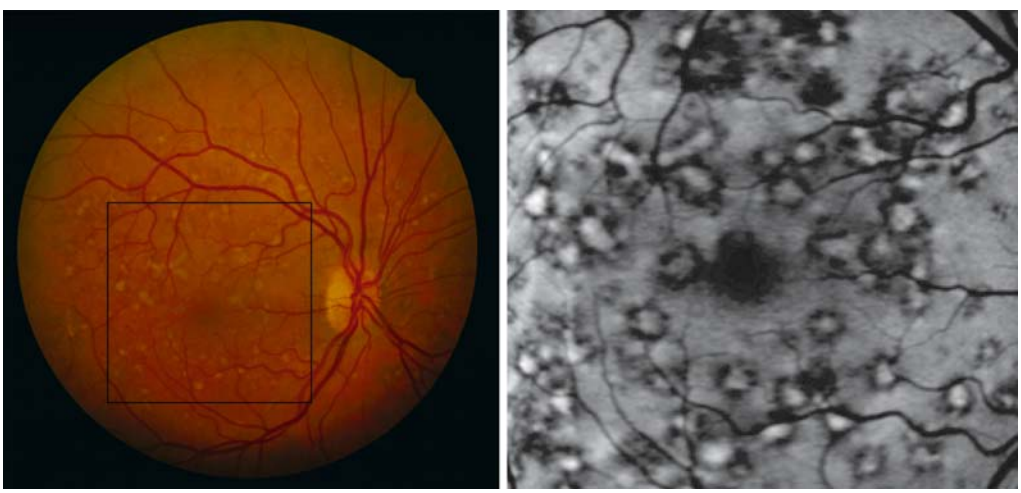

Fig. 2.8. Fundus photograph (*left*) and FAF mean image (*right*) of a patient with Kjellin's syndrome. Funduscopically visible multiple round yellowish flecks at the level of the RPE appeared as spots with increased FAF in the centre and with a halo of reduced autofluorescence

FAF in the centre, with a halo of reduced autofluorescence (Fig. 2.8).

Very recently, Lorenz et al. evaluated FAF in patients with early-onset severe retinal dystrophy (EOSRD) associated with mutations on both alleles of *RPE65*. They found absent or minimal FAF in all patients with compound heterozygous or homozygous *RPE65* mutations and concluded that lack of FAF in these patients is in accordance with the biochemical defect and can be used as a clinical marker of this geonotype [34].

Scholl et al. investigated whether the photoreceptor/RPE complex is still viable in patients that are blind from Leber congenital amaurosis (LCA). They found that in a subgroup of LCA patients FAF can be normal. This finding suggests that there is continuous metabolic demand from the photoreceptors and that the RPE/photoreceptor complex is, at least in part, anatomically intact, but the photoreceptors have lost function. This indicates that with future treatment modalities photoreceptor function may still be rescuable in such patients [47].

Summary for the Clinician

- **FAF is a valuable diagnostic tool for the further characterization of macular and retinal dystrophies**
- **In Best disease, adult vitelliform macular dystrophy and fundus flavimaculatus funduscopically visible yellowish deposits are associated with markedly increased autofluorescence intensity**
- **In patients with Stargardt macular dystrophy-fundus flavimaculatus, normal or low levels of FAF may be associated with peripheral photoreceptor dysfunction**
- **FAF imaging may be a more suitable diagnostic imaging device for following patients with hereditary retinal degenerations when compared with conventional fundus photography**

2.3 Further Applications

2.3.1 Automated Detection of Geographic Atrophies

As areas of geographic atrophy are readily delineated in FAF images, the affected areas can be precisely measured in digital FAF images. This may be particularly helpful in longitudinal analyses as well as for monitoring effects of future therapeutic interventions to slow down enlargement and, thus, visual loss from geographic atrophy. A recently published automated quantification procedure used customized imaging analysis software to facilitate detection and measurement of atrophic areas [43]. Although this method is more precise compared to a mouse-driven manual outlining of atrophic patches, it requires export of images, and manual 'whitewashing' of retinal vessels that are in contact with the atrophic patch as these are also associated with decreased FAF signal due to blockage of the FAF signal. Finally, the data had to be transferred into data processing software. An improved approach has therefore been developed which applies different image processing operators and an algorithm to detect retinal vessels automatically (Fig. 2.9) [16].

Summary for the Clinician

- **Areas of geographic atrophy can be accurately delineated in FAF images**
- **Software development allows for automated detection of areas of atrophy in digital FAF images and, thus, facilitation of quantitative analyses of spread of geographic atrophy over time**

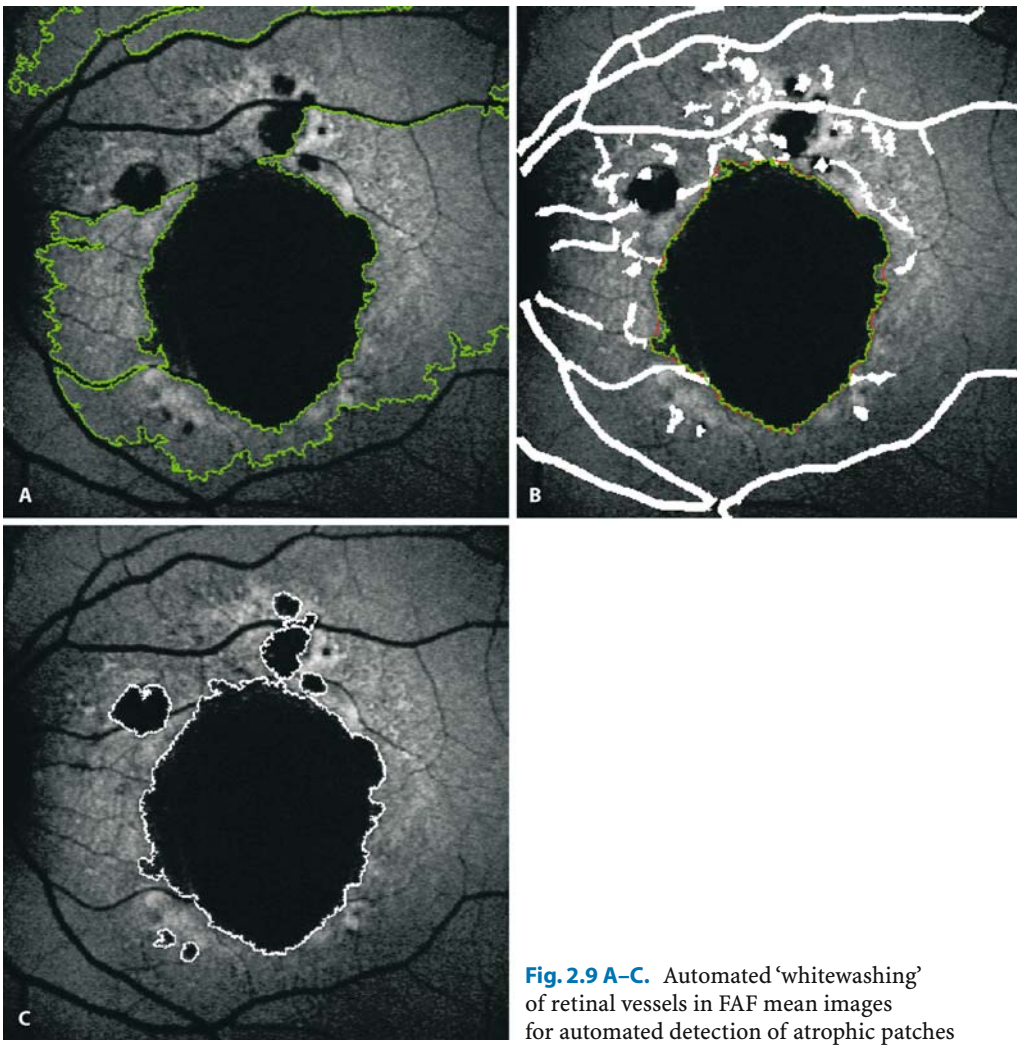

Fig. 2.9 A–C. Automated 'whitewashing' of retinal vessels in FAF mean images for automated detection of atrophic patches

2.3.2 Macular Pigment Density and Distribution

The yellow macular pigment with its compounds lutein and zeaxanthin has antioxidant and short wavelength absorbing properties. It protects the macular neurosensory retina and the RPE against oxidative damage. It has therefore been hypothesized that a decreased macular pigment density (MPD) may serve as a risk factor for the development and progression of AMD. Likewise supplementation with lutein and zeaxanthin may help to increase MPD and may have a prophylactic effect [6, 38]. Previous methods for quantifying macular pigment density include heterochromatic flicker photometry and motion photometry [39]. These require active participation of the examined patient. In contrast, FAF imaging with a confocal scanning laser ophthalmoscope allows for objective recordings of MPD measurements and determination of the distribution of MP [37]. While this is already possible with a single excitation wavelength of

488 nm, the use of two different wavelengths and subsequent subtraction may be more accurate [58]. Hereby FAF images of the posterior pole are obtained at 488 nm and 514 nm with a band-pass filter at 530 nm. MPDs are quantified by calculation of an MPD map and comparing foveal and parafoveal FAF at the two wavelengths. The MPD is created by digital subtraction of the log FAF images. MPD maps are then processed to calculate MPD within a 2° diameter circle centred on the fovea. The advantage of this approach over previous techniques besides its objective determination is that the examination requires very little time and that it is characterized by a high reproducibility.

Summary for the Clinician

- **Macular pigment density measurements and recording of its topographic distribution can be achieved with FAF imaging**
- **The measurements are based on the short-wavelength absorbing properties of the two macular pigment compounds lutein and zeaxanthin**
- **The advantage of this approach over previous psychophysical techniques besides its objective determination is that the examination requires very little time and that it is characterized by a high reproducibility**

2.3.3 High-Resolution In Vivo Fundus Autofluorescence Imaging

Advances in ocular imaging such as optical coherence tomography (OCT) and the use of adaptive optics allow for visualization of anatomical structures that have been unidentifiable with previous imaging methods [20, 33]. The delineation of single RPE cells in vivo, however, has not yet been achieved until recently. Based on the particular distribution of lipofuscin granules in the RPE cell cytoplasm, which are more dense at the lateral cell borders, visualization and delineation of RPE cells became possible using high-resolution FAF imaging. Using a new generation cSLO (Heidelberg Retina Angiograph 2, Heidelberg Engineering) with a theoretical horizontal resolution of up to 5 µm, the polygonal RPE cell layer in the presence of clear optical media has recently been visualized [10]. Interestingly, individual RPE cells show a wide variation in lipofuscin-dependent FAF intensity (Fig. 2.10). This technique will be useful in determining morphological and lipofuscin-dependent alterations in retinal diseases and may be applicable for monitoring effects of therapeutic interventions which target the RPE. A further improvement in resolution of FAF images would be expected from the combination of adaptive optics and the current cSLO-imaging technique.

Summary for the Clinician

- **High-resolution fundus autofluorescence imaging with improvements in confocal scanning laser ophthalmoscopy technology now allows for visualization of individual polygonal RPE cells in the living eye**
- **Both morphological changes and the highly variable lipofusin content of individual cells may be monitored during the natural course of retinal diseases and following interventions targeting the retinal pigment epithelium**

2.4 Summary

Ophthalmic imaging technology has revolutionized fundus examination. FAF imaging represents one of various novel tools and provides information over and above

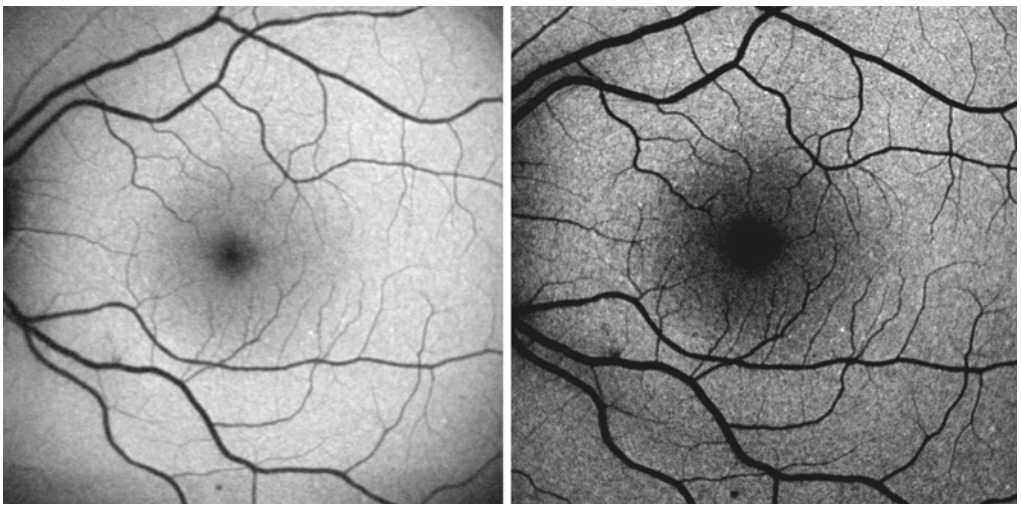

Fig. 2.10. FAF mean image of a 67-year-old patient taken with a confocal scanning laser ophthalmoscope (*left* HRA classic, *right* HRA 2). Due to the high resolution the right FAF image visualizes the polygonal RPE cell pattern that derives from intracytoplasmic lipofuscin granules

fundus photography, fluorescence angiography and optical coherence tomography. This noninvasive diagnostic tool visualizes age- and disease-related metabolic changes of the retinal pigment epithelium. The autofluorescence signal mainly derives from dominant fluorophores in lipofuscin granules of the RPE. Lipofuscin accumulation represents a common downstream pathogenetic pathway in many retinal and macular disease entities. Thus FAF imaging contributes significantly to our understanding of the pathophysiology and treatment of various retinal diseases.

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